इंटरनेट मानक

Disclosure to Promote the Right To Information

Whereas the Parliament of India has set out to provide a practical regime of right to information for citizens to secure access to information under the control of public authorities, in order to promote transparency and accountability in the working of every public authority, and whereas the attached publication of the Bureau of Indian Standards is of particular interest to the public, particularly disadvantaged communities and those engaged in the pursuit of education and knowledge, the attached public safety standard is made available to promote the timely dissemination of this information in an accurate manner to the public.

"जानने का अधिकार, जीने का अधिकार"
Mazdoor Kisan Shakti Sangathan
"The Right to Information, The Right to Live"

"पुराने को छोड नये के तरफ"
Jawaharlal Nehru
"Step Out From the Old to the New"

IS 14628 (1999): Method for Determination of Chlorinated Pesticide Residues in Agricultural and Food Commodities by Multiple Residues Technique [FAD 1: Pesticides and Pesticides Residue Analysis]

"ज्ञान से एक नये भारत का निर्माण"
Satyanarayan Gangaram Pitroda
"Invent a New India Using Knowledge"

Act Number 22 of 2005
The Indian Parliament
RIGHT TO INFORMATION

Made Available By
Public.Resource.Org

"ज्ञान एक ऐसा खजाना है जो कभी चुराया नहीं जा सकता है"
Bhartṛhari—Nītiśatakam
"Knowledge is such a treasure which cannot be stolen"

BLANK PAGE

BRUTUM FULMEN

IGNORANTIA JURIS NON EXCUSAT

PROTECTED BY COPYRIGHT

IS 14628 : 1999
(Reaffirmed 2004)

भारतीय मानक

कृषि खाद्य पदार्थों में क्लोरीनेटेड कीटनाशक अवशेष ज्ञात करने की पद्धति

Indian Standard

METHOD FOR DETERMINATION OF CHLORINATED PESTICIDE RESIDUES IN AGRICULTURAL AND FOOD COMMODITIES BY MULTIPLE RESIDUES TECHNIQUE

ICS 65.100; 67.040

© BIS 1999

BUREAU OF INDIAN STANDARDS
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002

February 1999

Price Group 2

Pesticides Residue Analysis Sectional Committee, FAD 34

FOREWORD

This Indian Standard was adopted by the Bureau of Indian Standards, after the draft finalized by the Pesticides Residue Analysis Sectional Committee had been approved by the Food and Agriculture Division Council.

Chlorinated pesticides such as BHC (HCH) and endosulfan are widely used as insecticide in agriculture. Earlier DDT was also used for the control of insects pests of agricultural importance which is now banned for use in agriculture but residues of the same are still present in agricultural and food commodities. This standard will enable the food, health authorities and other engaged in the field to follow uniform test procedure for the estimation of residues of BHC (HCH) and its various isomers (alpha, beta, gamma, delta), DDT and its isomers (P.P′ DDE, pp′ TDE — o, p′ DDE) and endosulfan and its metabolites (endosulfan alpha, endosulfan beta and endosulfan sulphate) in agriculture and food commodities.

In the preparation of this standard, due consideration has been given to the limits of BHC (HCH) and its isomers (alpha, beta, gamma, delta), DDT and its isomers (P.P′ DDE, pp′ TDE — o, p′ DDE) and endosulfan and its metabolites (endosulfan alpha, endosulfan beta and endosulfan sulphate) laid down under the provisions of *Prevention of Food Adulteration Act*, 1954 and Rules framed thereunder.

In reporting the results of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS 2 : 1960 ‘Rules for rounding off numerical values (*revised*)’.

Indian Standard

METHOD FOR DETERMINATION OF CHLORINATED PESTICIDE RESIDUES IN AGRICULTURAL AND FOOD COMMODITIES BY MULTIPLE RESIDUES TECHNIQUE

1 SCOPE

This standard prescribes gas chromatographic method for determination of residues of organochlorine compounds like BHC (HCH) and its various isomers (alpha, beta, gamma and delta), DDT and its degradation products or metabolites (P.P′ DDE, pp′ TDE — o, p′ DDE and o, p TDE) and endosulfan and its metabolites (endosulfan alpha, endosulfan beta and endosulfan sulphate) in agricultural and food commodities.

2 REFERENCE

The following Indian Standard contains provisions which through reference in this text, constitutes provision of this standard. At the time of publication, the edition indicated was valid. All standards are subject to revision and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent edition of the standard indicated below:

IS No.	*Title*
11380 (Part 1) : 1985	Method of sampling for the determination of pesticide residues : Part 1 Agricultural and food commodities

3 SAMPLING

The representative samples for the purpose of estimating BHC (HCH) and its various isomers, DDT and its degradation products or isomers residues and endosulfan and its metabolites in the materials shall be drawn in accordance with IS 11380 (Part 1).

4 PRINCIPLE

High moisture food material are thoroughly mixed and extracted with acetonitrite, whereas low moisture food materials are extracted with aqueous acetonitrile. Aliquot of acetonitrile is diluted with water and residues are extracted into *n*-hexane, *n*-hexane solution from various substracts is cleaned up by treatment with concentrated sulphuric acid. In case of endosulfan the clean up is carried out by using *Darco G 60* (charcoal). The insecticides residues are then estimated by gas liquid chromatography equipped with electron capture detector.

5 SOLVENT PURITY TEST

Solvents must be purified and final distillation conducted in all glass apparatus.

5.1 Acetonitrile

To 1 litre acetonitrile add 1 ml phosphoric acid, and 10 g phosphorous penta oxide and boiling chips and distil in all glass apparatus at 81-82°C.

5.2 Acetonitrile Saturated Petroleum Ether

Saturated with redistilled *n*-hexane or petroleum ether.

5.3 Concentrated Sulphuric Acid

5.4 GLC equipped with electron capture detector requires absence of substances causing detector response as indicated by the following test. Place 300 ml solvent A in Kuderna-Danish concentrator fitted with 3-boll synder column and collaborate collection vessel, and evaporate to 5 ml. Inject 1-2 µl concentrate by 10 µl syringe into gas chromatograph using conditions described in the method. Concentrate must not cause recorder deflection 1 mm from base line for 2-60 minutes after injection.

5.5 Darco G 60 (Activated Charcoal)

6 APPARATUS

6.1 Gas Liquid Chromatograph

Gas liquid chromatograph equipped with electron capture detector and printer plotter cum integrator. The following operating conditions are suggested, which may be changed, provided standardization is done.

Column: Packed with 1.5 percent OV-17 + 1.95 percent OV-210 liquid phase coated in gas chrom Q (80-120 mesh) or 0.50 percent OV-210 on gas chrom Q (80-100 mesh).

Temperatures:

Injection port temperature 210°C

Column oven temperature 190°C
Detector temperature 220°C
Carrier gas ultra-pure, nitrogen or equivalent
Gas flow rate 30 ml/minute

6.1.1 Extraction column for cleanup as in Fig. 1.

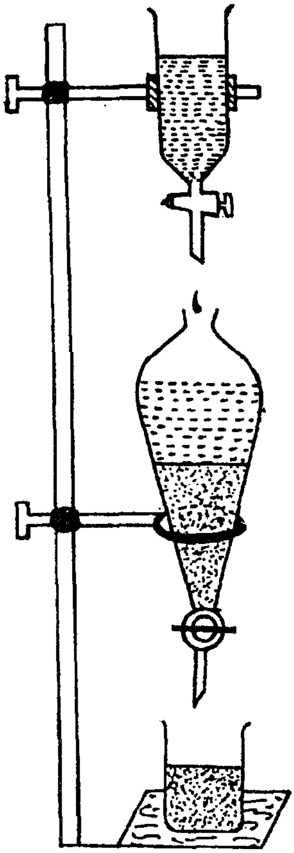

FIG. 1 CLEANUP APPARATUS

6.1.2 Stoppered cylinders of 100 ml capacity (Length of colour 6 × 0.25" dimension).

6.1.3 *Rotary Vacuum Evaporator*

6.1.4 *High Speed Blender*

6.1.5 *Kuderna-Danish Evaporator*

6.1.6 *Separating Funnels*, 1 000 and 125 ml with teflon stopcocks.

6.2 Reagents

6.2.1 *Acetonitrile*

6.2.2 *Acetonitrile*

Aqueous of water (65 to 35).

6.2.3 *n-Hexane*

6.2.4 *Acetone*

6.2.5 *Sodium Chloride*

Saturated solution.

6.2.6 *Sodium Sulphate*

Anhydrous.

6.2.7 *Sodium Sulphate*

6.2.8 *Sulphuric Acid*

Concentrated.

6.2.9 *Propylene Glycol*

6.2.10 *Distilled Water*

6.3 Preparation of Working Standards

6.3.1 Prepare working standard of (10 µg/ml) each of alpha, beta, gamma and delta isomers of BHC, DDT and its isomers metabolites (P.P′ DDE, pp′ TDE — o, p′ DDE and o, p TDE) and endosulfan and its metabolites (endosulfan alfa, endosulfan beta and endosulfan sulphate) of each solution.

6.3.2 For fortification — prepare standard mixture containing 1 µg/ml each of the above pesticides DDT and BHC. Prepare separate standard mixture (1 µg/ml) of endosulfan alfa, endosulfan beta and endosulfan sulphate.

7 EXTRACTION

7.1 Material of high moisture content of 50 g (vegetables and fruits) are chopped. Chopped material is then taken in to high speed blender jar, add 100 ml acetonitrile and approximately 10 g celite and blend 2 minute at high speed.

Filter through suction into 500 ml suction flask. Repeat the process with the sample. Collect the acetonitrile layer add 100 ml of *n*-hexane, 10 ml solution of saturated sodium chloride and 600 ml of distilled water. Shake vigorously for 45-50 seconds. Separate the aqueous layer and again extract twice with 2 × 50 ml *n*-hexane. Mix the *n*-hexane and concentrate in the Kuderna-Danish evaporator using a drop of propylneglycol 250 ml.

7.2 Pulses, Rice, Spices and Oilseeds

Materials having low moisture content of 50 g, such as pulses, rice, spices and oilseeds are grounded and extracted with aqueous acetonitrile as mentioned in **7.1**.

7.3 Milk Samples

7.3.1 Either **7.3.1.1** and **7.3.1.2** may be followed for extraction of the pesticide in milk samples.

7.3.1.1 Place 100 ml of milk in a 250 ml centrifuge bottle. Add 30 ml potassium oxalate solution and warm to about 45°C on a water bath. Add 10 ml aqueous mixture of Triton X-45, mix thoroughly and maintain at 45°C for 20 minutes. Cool to room temperature. Centrifuge to obtain a clear fat layer and add 100 ml of *n*-hexane. Stopper and shake the bottle gently to gather the fat layer. Centrifuge until the *n*-hexane layer is clear. Remove the *n*-hexane layer without disturbing the milky phase. To wash over the *n*-hexane left behind, slowly add another 100 ml of *n*- hexane (do not disturb the milky phase), separate and add to the first extract. Give one more washing and combine all the extracts. Filter the extract in Kuderna-Danish evaporator and concentrate under the nitrogen atmosphere to dryness (or fatty residues).

7.3.1.2 Add acetone to 100 ml of milk which will result in coarse precipitation of milk solids. Remove the solids by filtering through glass wool. Take the filterate in a separatory funnel and extract the pesticide twice with 100 ml of petroleum ether each time. Combine the extracts and concentrate it on water bath.

7.4 Butter

Warm the butter at 50°C until fat separates. Decontent fat through dry filter and take 5 g of clean fat sample and proceed as mentioned in **7.3**.

7.5 Milk Powder

Take 9 g of milk powder and add 100 ml of warm distilled water shake well to ensure homogeneity and proceed as mentioned in **7.3**.

8 CLEANUP

8.1 Cleanup of BHC (HCH) and its isomers (alpha, beta, gamma, delta) and DDT and its metabolites (P.P′ DDE, pp′ TDE — o, p′ DDE).

Take *n*-hexane in an extraction column (Fig. 1) and add dropwise about 40 ml of concentrated sulphuric acid. Discard lower phase. Wash the upper *n*-hexane layer three times with 40 ml portions of distilled water. Dry it by passing through 50 g anhydrous sodium sulphate followed with 10 ml of *n*-hexane. Concentrate the combined *n*-hexane extract to 5 ml. Estimate the BHC (HCH) and DDT residues by GLC.

8.2 Cleanup of endosulfan alfa, endosulfan beta and endosulfan sulfate.

Take 20 ml of *n*-hexane extract. Add 2.5 ml of acetone and 0.50 ml of *Darco g 60*, shake it and filter. Take filtrate in an extraction column (Fig. 1) and add dropwise about 40 ml of concentrated sulphuric acid. Discard lower phase, wash the upper *n*-haxane layer three times with 40 ml of distilled water. Dry it by passing through 5 g anhydrous sodium sulphate. Make the volume of residues to 2 ml with *n*-hexane: acetone (19:1) (*v*/*v*). Estimate endosulfan and its metabolites by GLC.

9 ESTIMATION

9.1 Estimation of Residues of BHC (HCH) and Its Isomers and DDT and Its Metabolites

Condition the GLC by giving several injections of standards simultaneously. Set operating parameters to obtain halfscale deflection at least with 0.5 mg p,p' — DDE and adequate resolution of the compounds of interest. Inject standard mixtures at two concentration levels to obtain 30 to 40 percent and 60 to 80 percent full scale deflection (FSD) for various compounds, and check variation due to non-linearity of electron capture detector. Inject suitable aliquots (2 to 10 µl) of the cleaned up extracts so that the injection represents about 20 mg sample equivalent. If required, dilute the extracts to get peak heights within the scale. Identify the compounds by comparison of retention times of peaks in sample chromatogram with those of standard compounds run under identical operating conditions. Use peak heights of standards that are similar (having less than 25 percent difference) to peaks in sample chromatograms to quantify the residues.

9.2 Estimation of Endosulfan and Its Metabolites

Condition the GLC as described in **9.1**. Inject the suitable aliquot (2 to 10 µl) of the cleaned up extract of endosulfan and identify the compounds by comparison of retention times of peaks in sample chromatogram with tose of standard compounds run under identical operating conditions. Use peak heights of standards that are similar (having less than 25 percent difference) to peaks in sample chromatograms to quantify the residues.

9.3 Calculation

$$\text{Residue level (mg/kg)} = \frac{\text{Peak height of the sample}}{\text{Peak height of the standard}} \times \frac{\mu\text{g of the standard injected}}{\mu\text{l of the sample extract injected}} \times \frac{\text{Final volume of the sample extract(ml)}}{\text{mass in g of the sample}}$$

9.4 Expression of Results

Report residue levels of each compound as well as the sums of isomers alpha, beta, gamma and delta BHC (HCH), p, p' DDE, p, p' TDE, and o, p 'DDE' and o,p 'TDE' and endosulfan alpha, endosulfan beta and endosulfan sulfate in mg/kg. If recoveries obtained are greater than 80 percent, residue levels should not be corrected for recovery values.

Quantify only those peaks which represent greater than 10 percent FSD when 20 mg sample equivalents are injected in GLC. When a peak is distinguishable from baseline but less than 10 percent FSD, report it as trace. If signal is not distinguishable from the background at the retention time of a compound, report it as not detectable.

Round off the residue values to two decimal points. Levels less than 0.01 mg/kg should also be reported as trace.

10 RECOVERY STUDIES

Perform recovery studies regularly to check the reliability of the analytical technique as well as variations due to handling procedure. Take two sets of three samples each of a commodity and fortify them at levels of 0.05 and 0.5 mg/kg for various residues, respectively. Analyse the fortified samples by following the applicable procedure. The recoveries of different compounds over the background values from unfortified samples should be above 80 percent of the added amounts. The difference between the residues found in the replicates should be less than 20 percent. It is desirable to select those samples for recovery studies which are having low levels of contamination. If residues in samples are high, increase the fortification level to about double of the background level.

11 CONFIRMATION OF THE RESIDUES

11.1 Determination of residues by GLC equipped with electron capture detector needs confirmation. It is not necessary to confirm the nature of residues in every sample. The residues may, however, be confirmed in one sample out of five. In addition, samples showing exceptionally high residues or unusual GLC pattern should also be subject to confirmatory procedure. Depending on the tentative nature and levels of residues one or more of the following confirmatory techniques may be used.

11.1.1 *Micro-alkali Derivatization*

Take 5 ml aliquot of cleared up *n*-hexane extract having residue concentrations suitable for GLC analysis in a 15 ml graduated tube. Add 2 ml ethanolic potassium hydroxide (5 percent) and heat in a water bath at 50°C for 2 hours. Add more *n*-hexane when the solution in the tube nears drying. Remove the tube from the water bath, cool and add 5 ml ethanol: water (1:1) (*v/v*) and 2 ml hexane, stopper and shake gently. Allow the layers to separate and inject suitable aliquots of upper *n*-hexane phase into GLC. Confirm disappearance of shifts in the peak pattern in alkali derivatized sample as compared to underivatized sample. By this method, p, p' — DDT, o, p' — DDT and p, p' - TDH will change to p, p 'DDE, o, p'DDE and DDMU, respectively, while p,p 'DDE will not be affected. The peak of BHC (HCH) isomers completely disappear after this treatment.

12 INTERFERENCE DUE TO POLYCHLORINATED BIPHENYLS

Polychlorinated biphenyls are industrial chemicals used in manufacture of synthetic rubber, electrical products, printing inks, paints, varnishes, resins, etc. These are resistant to oxidation and reduction and thus, like DDT residues, are widely distributed in the environment. Being polychlorinated biphenyls, these have physical and chemical properties of chlorinated hydrocarbon insecticide residues and thus a method of analysis of DDT and BHC (HCH) will also detect polychlorinated biphenyls. In addition to the environmental contamination of the sample, polychlorinated biphenyls from rubber and plastic caps and lining of the containers and laboratory apparatus may also cause interferences. On GLC, polychlorinated biphenyls produce multiple peaks some of which may have same retention times as that of BHC (HCH) and DDT residues. So due care should be taken of BHC (HCH) and DDT residues.

If a sample gives multiple peaks on GLC [as will be in the case with sample containing both BHC (HCH) and DDT residue]. The absence or presence of polychlorinated biphenyl may be confirmed by micro-alkali treatment.

Bureau of Indian Standards

BIS is a statutory institution established under the *Bureau of Indian Standards Act,* 1986 to promo harmonious development of the activities of standardization, marking and quality certification of goo and attending to connected matters in the country.

Copyright

BIS has the copyright of all its publications. No part of these publications may be reproduced in any form without the prior permission in writing of BIS. This does not preclude the free use, in the course of implementing the standard, of necessary details, such as symbols and sizes, type or grade designations. Enquiries relating to copyright be addressed to the Director (Publications), BIS.

Review of Indian Standards

Amendments are issued to standards as the need arises on the basis of comments. Standards are also reviewed periodically; a standard along with amendments is reaffirmed when such review indicates that no changes are needed; if the review indicates that changes are needed, it is taken up for revision. Users of Indian Standards should ascertain that they are in possession of the latest amendments or edition by referring to the latest issue of 'BIS Handbook' and 'Standards: Monthly Additions'.

This Indian Standard has been developed from Doc : No. FAD 34 (490).

Amendments Issued Since Publication

Amend No.	Date of Issue	Text Affected

BUREAU OF INDIAN STANDARDS

Headquarters:

Manak Bhavan, 9 Bahadur Shah Zafar Marg, New Delhi 110 002
Telephones : 323 01 31, 323 33 75, 323 94 02

Telegrams : Manaksanstha
(Common to all offices)

Regional Offices :

		Telephone
Central	: Manak Bhavan, 9 Bahadur Shah Zafar Marg NEW DELHI 110 002	323 76 17 323 38 41
Eastern	: 1/14 C. I.T. Scheme VII M, V. I. P. Road, Kankurgachi CALCUTTA 700 054	337 84 99, 337 85 61 337 86 26, 337 91 20
Northern	: SCO 335-336, Sector 34-A, CHANDIGARH 160 022	60 38 43 60 20 25
Southern	: C. I. T. Campus, IV Cross Road, CHENNAI 600 113	235 02 16, 235 04 42 235 15 19, 235 23 15
Western	: Manakalaya, E9 MIDC, Marol, Andheri (East) MUMBAI 400 093	832 92 95, 832 78 58 832 78 91, 832 78 92

Branches : AHMADABAD. BANGALORE. BHOPAL. BHUBANESHWAR. COIMBATORE. FARIDABAD. GHAZIABAD. GUWAHATI. HYDERABAD. JAIPUR. KANPUR. LUCKNOW. NAGPUR. PATNA. PUNE. RAJKOT. THIRUVANANTHAPURAM.

Printed at : Prabhat Offset Press. New Delhi-2